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Research Article

ACUTE TOXICITY STUDY OF *VISHATINDUKADI VATI* PREPARED BY TWO REFERENCES: EVALUATION OF TOXIC EFFECTS IN WISTAR RATS

Rimpal Patel[1]*, Kirti Patel[2], Bharat Kalsariya[3]

*[1]PG Scholar, [3]Professor & Principal Upgraded Department of Rasashastra & Bhaishajya Kalpana, Government Ayurved College, Vadodara.

[2]Dean (I/C) & Head of the Department, Faculty of Pharmacy, The M.S. University, Vadodara, Gujarat, India.

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ABSTRACT

Acute toxicity studies were conducted to assess the short-term adverse effects of two formulations of *Vishatindukadi Vati* (VTV1 and VTV2) in rats, as per OECD 423 guidelines. VTV1 contains *Kupilu, Maricha, Puga Phala* and *Chincha Beeja* while VTV2 contains *Kupilu, Maricha* and *Bhavana* of *Nagavalli Patra Svarasa*. This study aims to conduct an acute toxicity study of *Vishatindukadi Vati*, prepared with two references. The experimental procedure involves using female Wistar rats (Rattus norvegicus), aged 8 weeks. Each rat weighs between 200 and 300 grams, with a variation of ± 20% of the mean weight. A total of 3 animals are used per set for the study. Mortality occurred in all animals administered VTV1 at 2000mg/kg body weight, whereas no mortality was observed in VTV2 at the same dose. VTV1 exhibited toxicity, potentially due to the high concentration of *Kupilu*, which contains the neurotoxic alkaloids strychnine and brucine. The *Bhavana* of *Nagavalli Patra* in VTV2, known for its antidote properties, may reduce oxidative stress and inflammation, potentially mitigating toxicity. The LD50 of VTV1 was classified under GHS Category 4 (greater than 300mg/kg), while VTV2 fell under GHS Category 5 or unclassified (greater than 2000mg/kg). This study emphasizes the significance of *Shodhana* (purification) processes and antidote substances in mitigating the toxicity of formulations.

INTRODUCTION

Acute toxicity studies are conducted to determine the short-term adverse effects of a drug when administered in a single dose or multiple doses during 24 hours in two mammalian species.[1] *Vishatindukadi Vati* is herbo-mineral formulation. In Rasatantrasaar and Siddhaprayoga Sangraha has mentioned two different references with different ingredients for the preparation of *Vishatindukadi Vati*. The formulation mentioned in *Gutika Prakarana* contains *Kupilu* (*Strychnos nux- vomica* Linn), *Maricha* (*Piper nigrum* Linn.), *Puga Phala* (*Araca catechu* Linn.) and *Chincha Bija* (*Tamarindus indica* Linn).[2] while the formulation mentioned in *Vatavyadhi Prakarana* contains *Kupilu* (*Strychnos nux-vomica* Linn.) and *Maricha* (*Piper nigrum* Linn.) with *Bhavana* of *Nagavalli* (*Piper betel* Linn.) *Patra Swarasa.*[3] Among all ingredients *Kupilu* is categorized in *Upavisha* (mild potency poisons) *Dravya*.

From toxicological views, both Ayurveda and modern texts explained its toxicity. It contains poisonous indole alkaloids strychnine (1.23% in seeds) and brucine (1.55% in seeds), pseudo-strychnine and iso strychnine. Strychnine has poisonous action on anterior horn cells of spinal cord.[4] *Vishatinduka Beeja* should always be used as medicine after doing proper *Shodhana* (purification process). There is a need for scientific revalidation for all such formulations which contain poisonous plant-based raw material. *Vishtindukadi Vati* is one such poisonous plant-based formulation. Hence, evaluation of acute toxicity profile of *Vishtindukadi Vati.*

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MATERIAL AND METHODS

Procurement and authentication of the raw material

Kupilu, *Maricha* and *Puga Phala* were procured from the Government Ayurved Pharmacy, Rajpipala, Gujarat, India. *Chincha Beeja* and *Nagavalli Patra* were

procured from the local market of Vadodara, Gujarat. *Eranda Taila* was procured from the local market of Vadodara, Gujarat, as per *fssai* standards.

Preparation of drug

All the batches of *Vishatindukadi Vati* 1 and *Vishatindukadi Vati* 2 were prepared in pharmaceutical laboratory of Upgraded Department of Rasashastra and Bhaishajya Kalpana, Government Ayurved College, Vadodara, Gujarat.

The batch of *Vishatindukadi Vati* 1 was prepared as per the reference of Rasatantrasaara and Siddhaprayog Sangraha, part 1, *Gutika Prakarana* contained *Kupilu, Maricha, Puga Phala* and *Chincha Beeja*.[5] The batch of *Vishatindukadi Vati* 2 was prepared as per the reference of Rasatantrasaara and Siddhaprayog Sangraha, part 2, *Vatavyadhi Prakrana* contains *Kupilu, Maricha* and *Bhavana* of *Nagavalli Patra Svarasa.*[6]

1. *Kupilu Shodhana*[7]

Eranda Taila was taken in s.s.vessel and heated slightly. *Ashuddha Kupilu* was added and *Bharjana* was done in *Eranda Taila* till it puffed up. After that, it was taken out from the vessel; the testa and embryo were removed with the knife. *Shuddha Kupilu* was collected and stored in airtight container. [Figure 1]

2. Preparation of *Churna*[8] of *Shuddha Kupilu, Maricha, Puga Phala* and *Chincha Beeja*

Shuddha. Kupilu, Maricha, Puga Phala and *Chincha Beeja* were crushed in mortar and pestle individually and ground in the mixer grinder. *Maricha, Puga Phala* and *Chincha Beeja* were sieved through #120 while *Shuddha Kupilu* sieved through #60. Sieved fine powders were collected and packed in air tight container.

3. Preparation of *Nagavalli Patra Svarasa*[9]

Fresh *Nagavalli* was taken in the above-mentioned quantity, washed with water and cleaned well. Then leaves were cut into small pieces with the help of a knife. After those small pieces were taken into a mixer grinder paste was prepared. *Svarasa* was obtained by squeezing the paste through the cotton cloth and measured. Collected *Svarasa* was used for further process. [Figure 2]

4. Preparation of *Vishatindukadi Vati* 1

Shuddha. Kupilu, Maricha, Puga Phala and *Chincha Beeja* were taken in the mortar pestle above mentioned quantity, trituration was carried out until it became a homogenous mixture. After proper mixing, water was added little by little and levigated well till became a doughy mass. After that, 240mg of *Vati* was prepared. *Vati* was shade-dried, weighted, labeled and stored in an airtight container. [Figure 3]

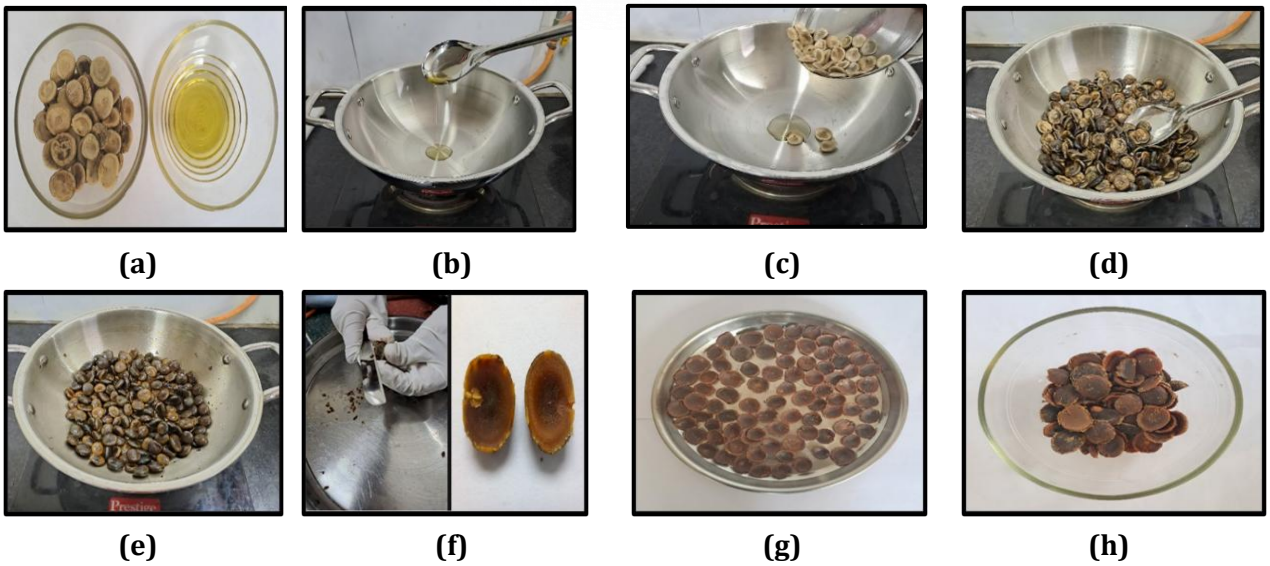
5.Preparation of *Vishatindukadi Vati* 2

Shuddha Kupilu and *Maricha* were taken in the mortar pestle as per above mentioned quantity, trituration was carried out until it became a homogenous mixture. After proper mixing, *Nagavalli Svarasa* was added little by little and continue levigated for 12 hours. After that, 240mg of *Vati* was prepared. *Vati* was shade-dried, weighted, labeled, and stored in an airtight container. [Figure 4]

Samples are Labeled as

Vishatindukadi Vati 1- VTV1

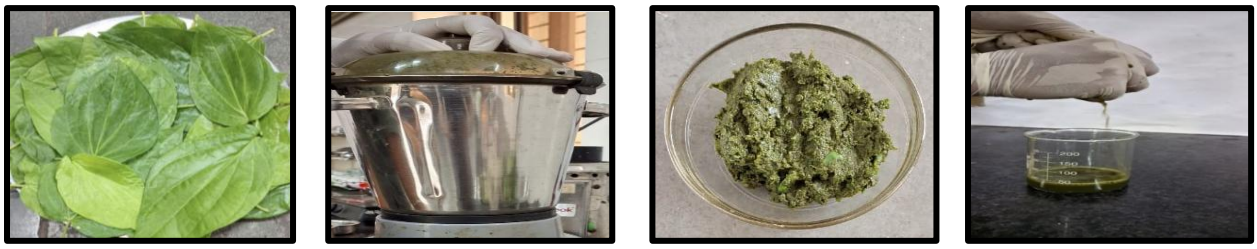
Vishatindukadi Vati 2- VTV2



(a) **(b)** **(c)** **(d)**

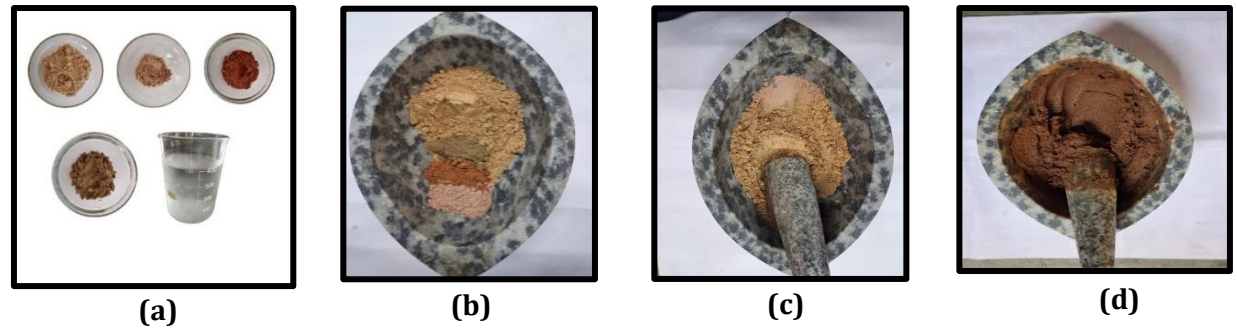
(e) **(f)** **(g)** **(h)**

Figure 1: (a) Ingredients **(b)** Taking *Eranda Taila* in s.s.vessel **(c)** Adding *Ashuddha Kupilu* in *Eranda Taila* **(d)** *Bharjana* of *Kupilu* **(e)** Puffed *Kupilu* **(f)** Removing testa and embryo **(g)** *Kupilu* after removing of embryo **(h)** *Shuddha Kupilu*



(A) **(B)** **(C)** **(D)**

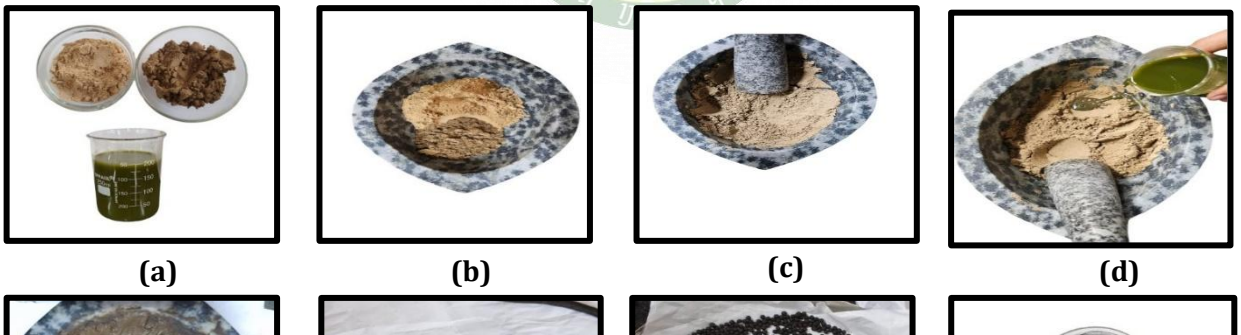
Figure 2: (a)*Nagavalli Patra* **(b)**Griding in mixer grinder **(c)** *Kalka* of *Nagavalli Patra* **(d)** Straining through cloth and *Nagavalli Patra Svarasa*



(a) **(b)** **(c)** **(d)**

(e) **(f)** **(g)** **(h)**

Figure 3: (a) Ingredients of *Vishatindukadi Vati* 1 **(b)** Taking ingredients in mortar **(c)** Homogenous mixture **(d)** After adding of Water in mixture **(e)** After levigation **(f)**Preparing *Vati* **(g)** Drying of pills **(h)** *Vishatindukadi Vati* 1



(a) **(b)** **(c)** **(d)**

(e) **(f)** **(g)** **(h)**

Figure 4: (a) Ingredients of *Vishatindukadi Vati* 2 **(b)** Taking ingredients in mortar **(c)** Homogenous mixture **(d)** After adding of *Svarasa* in mixture **(e)** After levigation **(f)** Preparing *Vati* **(g)** Drying of *Vati* **(h)** *Vishatindukadi Vati* 2

Study Information

The study, identified by the number RRC/NG/24/PT/0084, was conducted at a test facility located at Sr. No. 261/1, Block No. 271, near Dhanvantary Pharmacy College, Kudsad, Ta: Olpad, Surat, Gujarat, India. The samples tested during the study were VTV1 and VTV2. The study followed the

OECD 423: 2001 guidelines for acute oral toxicity testing, utilizing the toxic class method.

Experimental Procedure

The species used in the study were Wistar rats (Rattus norvegicus), specifically female animals. The rats were 8 weeks of age and weighed between 200-300 g, with a variation of ±20% of the mean weight. A total of 3 animals were used per set. The animals were housed under controlled environmental conditions, with a temperature range of 21.4-22.9°C, relative humidity between 47-64%, and a 12-hour light/dark cycle. They were acclimatized for 5 days prior to the start of the experiment. Each animal was identified using a marker pen, and the study followed the oral gavage method, as recommended by OECD Test Guideline 423. The test item was administered orally via gavage, with RO water used as the vehicle based on the solubility of the test item. A single dose of the test item was administered within a 24-hour period using an appropriate intubation cannula.

Table 1: Details of Treatment Procedure

Name of test item	Set	Route	Dose (mg/kg B.wt.)	Animal Identification Number
VTV1	I	Oral	300	1-3
	II	Oral	300	7-9
	III	Oral	2000	13-15
VTV2	I	Oral	300	4-6
	II	Oral	300	10-12
	III	Oral	2000	16-18
	IV	Oral	2000	22-24

Test Item Administration

Two formulations of *Vishatindukadi Vati* were tested in the study. For VTV1, the initial dose of 300mg/kg body weight was administered to Set I and Set II, followed by a higher dose of 2000mg/kg in Set III. For VTV2, the same dosing regimen was followed as for VTV1, with the addition of a confirmatory dose of 2000mg/kg in Set IV.

Rationale for Dose Selection

As there was no prior public domain information about these formulations, the starting dose as set at 300mg/kg body weight, with a subsequent higher dose of 2000mg/kg body weight for safety.

OBSERVATIONS

Mortality/Morbidity

No mortality was observed in any of the animals in Sets I and II, both of which were treated with a dose of 300mg/kg body weight of the test item VTV1. Mortality occurred in all three animals of Set III treated with 2000mg/kg body weight of the test item VTV1. No mortality was observed in any of the animals in Sets I, II, III and IV when treated with the test item VTV2.

Table 2: Individual Animal Mortality/Morbidity Observation of VTV1

Animal No.	Sex	Observation	Days														
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	F	1st															
		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	F	1st	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	F	1st	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	F	1st	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	F	1st	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	F	1st	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	F	1st	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	F	1st	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	F	1st	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	F	1st	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	F	1st	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	F	1st	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	F	1st	-	NA													
		2nd	D														
14	F	1st	-	NA													
		2nd	D														
15	F	1st	-	NA													
		2nd	D														

Table 3: Individual Animal Mortality/Morbidity Observation of VTV2

Animal No.	Sex	Observation	Days														
1	F	1st	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	F	1st	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	F	1st	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	F	1st	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	F	1st	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	F	1st	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	F	1st	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	F	1st	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	F	1st	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	F	1st	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	F	1st	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	F	1st	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	F	1st	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	F	1st	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	F	1st	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		2nd	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Behavioral Sign Observation

No abnormal clinical signs were detected in any of the animals in sets I and II. All three animals in Set III exhibited tremors with clinical signs observed after treatment with VTV1. No clinical signs were observed in any of the animals in Sets I, II, III and IV when treated with the test item VTV2.

Table 4: Individual Animal behavioral Sign observation Test item VTV1

Animal No.	Sex	Days																	
		0					1	2	3	4	5	6	7	8	9	10	11	12	13	14
		With in 30 min	1h	2h	3h	4h														
			± 10 minutes																	
1	F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3	F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	F	1	113, 2	NA																
14	F	1	113, 2	NA																
15	F	1	113, 2	NA																

Normal, F= Female, NA= Not Applicable, 2= Mortality 113= Tremor (Continuous, Repetitive Twitching (contraction) of Skeletal Muscle, which is usually visible and Palpable. the Movements are Rhythmic and Oscillatory.)

Table 5: Individual Animal behavioral Sign Observation Test item VTV 2

Animal No.	Sex	Days																	
		0					1	2	3	4	5	6	7	8	9	10	11	12	13	14
		With in 30 min	1h	2h	3h	4h														
			± 10 minutes																	
4	F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
22	F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
23	F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
24	F	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Body Weight

Body weight was observed within the normal range before the treatment. All surviving animals had normally gained body weight compared to day 1.

Table 6: Individual Animal Body Weight Record Test item VTV1

Animal No.	Sex	Dose (mg/kg b.wt.)	Amount of dose Administered (mL)	Body Weight (g)		
				Day 0	Day 7	Day 14
1	Female	300	2.5	249.7	263.1	279.3
2	Female		2.3	227.7	243.9	258.6
3	Female		2.3	227.1	244.1	261.9
7	Female	300	2.1	206.3	222.7	239.6
8	Female		2.4	243.8	256.3	273.2
9	Female		2.3	228.3	246.2	261.5
13	Female	2000	2.3	227.4	NA	NA
14	Female		2.3	228.0	NA	NA
15	Female		2.3	226.1	NA	NA

Table 7: Individual Animal Body Weight Record Test Item VTV 2

Animal No.	Sex	Dose (mg/kg b.wt.)	Amount of dose Administered (mL)	Body Weight (g)		
				Day 0	Day 7	Day 14
4	Female	300	2.3	227.8	243.1	256.2
5	Female		2.1	211.1	227.3	244.1
6	Female		2.1	213.1	231.7	248.6
10	Female	300	2.3	231.7	244.3	259.6
11	Female		2.2	217.0	232.9	248.7
12	Female		2.2	223.3	238.4	253.9
16	Female	2000	2.2	222.3	234.5	251.1
17	Female		2.3	232.6	245.9	263.8
18	Female		2.1	211.2	226.9	243.5
22	Female	2000	2.2	218.3	233.6	249.5
23	Female		2.5	245.0	261.3	276.8
24	Female		2.4	236.2	253.9	268.7

Gross Necropsy

No abnormalities were found in any of the animals from Set I and II that were terminally sacrificed after treatment with VTV1. In Set III, all animals were found dead with the test item present in their stomach, stomach became tympanic. No abrasions were observed in the oesophagus or trachea, and all other organs appeared normal. No abnormalities were found in any of the animals from Set I, Set II, III and IV that were terminally sacrificed after treatment with VTV2.

Table 8: Individual Animal Gross Pathology Examinations Test item VTV1

Animal No.	Sex	Mode of Death	Gross Pathology Observations
1	Female	Terminal Sacrifice	No Abnormality Detected
2	Female	Terminal Sacrifice	No Abnormality Detected
3	Female	Terminal Sacrifice	No Abnormality Detected
7	Female	Terminal Sacrifice	No Abnormality Detected
8	Female	Terminal Sacrifice	No Abnormality Detected
9	Female	Terminal Sacrifice	No Abnormality Detected

13	Female	Found Dead	Test item present in their stomach, stomach become tympanic. No abrasions were observed in the esophagus or trachea, and all other organs appeared normal.
14	Female	Found Dead	Test item present in their stomach, stomach become tympanic. No abrasions were observed in the esophagus or trachea, and all other organs appeared normal.
15	Female	Found Dead	Test item present in their stomach, stomach become tympanic. No abrasions were observed in the esophagus or trachea, and all other organs appeared normal.

Table 9: Individual Animal Body Weight Record of test item VTV2

Animal No.	Sex	Mode of Death	Gross Pathology Observations
4	Female	Terminal Sacrifice	No Abnormality Detected
5	Female	Terminal Sacrifice	No Abnormality Detected
6	Female	Terminal Sacrifice	No Abnormality Detected
10	Female	Terminal Sacrifice	No Abnormality Detected
11	Female	Terminal Sacrifice	No Abnormality Detected
12	Female	Terminal Sacrifice	No Abnormality Detected
16	Female	Terminal Sacrifice	No Abnormality Detected
17	Female	Terminal Sacrifice	No Abnormality Detected
18	Female	Terminal Sacrifice	No Abnormality Detected
22	Female	Terminal Sacrifice	No Abnormality Detected
23	Female	Terminal Sacrifice	No Abnormality Detected
24	Female	Terminal Sacrifice	No Abnormality Detected

DISCUSSION

In the VTV1 sample, mortality was observed (found dead 3 out of 3) at the dose of 2000mg/kg body weight. Conversely, in the VTV2 sample, no mortality was observed at the dose of 2000mg/kg body weight.

The toxicity and potential mortality observed in animals administered VTV1 can be attributed to Nux Vomica, a component containing the neurotoxic alkaloids strychnine and brucine. Strychnine acts as a competitive antagonist of glycine receptors in the central nervous system, inhibiting glycine's inhibitory action and increasing neuronal excitability. This hyperstimulation leads to continuous muscle contractions and tremors, as observed in the study, and can result in tetanic contractions, particularly in respiratory muscles, causing respiratory failure and contributing to mortality. Nux Vomica can also induce gastrointestinal distress, evident from tympanic distension of the stomach during necropsy, further exacerbating physiological stress.

In a previous study, *Kupilu* underwent *Shodhana* using *Bharajana* in *Eranda Taila*. HPTLC analysis of raw *Kupilu* revealed 1.44% strychnine and 0.66% brucine, which were reduced to 0.47% and 0.35%, respectively, after *Shodhana*. This reduction likely results from the conversion of strychnine and brucine into less toxic derivatives like isostrychnine, isobrucine, Strychnine N-oxide, and Brucine N-oxide.[10]

In the present study, *Kupilu Shodhana* was conducted following the RTSSPS method, as adopted in the previous research. Based on the data from this study, the concentrations of strychnine and brucine in VTV1 and VTV2 were calculated and converted into human equivalent doses using the CSIR dose calculation method. The percentage of *Shuddha Kupilu* in VTV1 81.63% and in VTV2 50%. *Shuddha Kupilu* in 70kg human (converted from 2000mg/kg) in VTV1 and VTV2 18.53g and 11.35 g respectively. Amount of Strychnine and Brucine in VTV1 is higher than in VTV2. Specifically, in a 70kg human, VTV1 contains 92.65mg of Strychnine and 64.85mg of Brucine, while VTV2 contains 56.75mg of Strychnine and 39.72mg of Brucine. This higher concentration of toxic compounds in VTV1 may explain the observed toxicity and potential mortality in rats following the administration of VTV1.

In VTV2, *Bhavana* of *Nagvalli Patra Svarasa* is mentioned whereas VTV1 is prepared by levigation of water. According to Aryabhishaka, *Nagavalli Patra* is used as an antidote for *Kupilu*.[11] The phytochemicals present in *Nagavalli Patra* may provide supportive effects in managing strychnine poisoning. Compounds

like tannin,[12] eugenol and hydroxychavicol possess antioxidant properties,[13,14] potentially helping to reduce oxidative stress associated with strychnine-induced convulsions. Eugenol's anti-inflammatory action may alleviate CNS inflammation caused by the toxin.[15] So, the *Bhavana* of *Nagavalli Patra Svarasa* may reduce the toxic effect of strychnine.

According to the reference, the percentage of *Kupilu* in VTV1 is 81.63%, while in VTV2 it is 50%, indicating that the concentration of *Kupilu* is higher in VTV1. Previous research has demonstrated that the *Shodhana* procedure effectively reduces the levels of strychnine and brucine. In VTV2, the *Bhavana* of *Nagvalli Patra Svarasa* is utilized, whereas VTV1 employs *Bhavana* with water. Notably, according to the *Aryabhishaka Nagvalli Patra Svarasa* has been documented as an antidote for *Kupilu*. The higher percentage of *Kupilu* in VTV1 may contribute to its toxicity, whereas combined with the *Bhavana* of *Nagvalli Patra Svarasa* in VTV2, may contribute to a reduction in the toxic effects observed in VTV2.

CONCLUSION

The test items, VTV1 and VTV2 were administered by oral route. The LD50 value of VTV1 was determined to be greater than 300mg/kg body weight, classifying it under GHS Category 4. The LD50 value of VTV2 was found to be greater than 2000mg/kg body weight, which classifies it under GHS Category 5 or as Unclassified. To further evaluate the toxicity profile, sub-acute and chronic toxicity studies can be conducted. Additionally, a clinical study can be performed to assess the clinical efficacy of *Vishatindukadi Vati*.

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***Address for correspondence**
Dr. Rimpal Patel
PG Scholar,
Upgraded Department of Rasashastra & Bhaishajya Kalpana,
Government Ayurved College,
Vadodara, Gujarat.
Email: drrimpalpatel29@gmail.com

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